Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2415–2418

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Substituted 3-Amino Biaryl Propionic Acids as Potent VLA-4 Antagonists

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Received 14 March 2002; accepted 20 May 2002

Abstract—A series of substituted *N*-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl- and (L)-azetidyl-β-biaryl β-alanine derivatives was prepared as selective and potent VLA-4 antagonists. The 2,6-dioxygenated biaryl substitution pattern is important for optimizing potency. Oral bioavailability was variable and may be a result of binding to circulating plasma proteins.

VLA-4 ($\alpha_4\beta_1$; CD49d/CD29; very late antigen-4) is a member of the integrin family that is expressed on lymphocytes.[1] Its ligands include vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated endothelial cells at sites of inflammation and peptides derived from the type III connecting segment (CS-1) domain of fibronectin. Antibodies to α4 are effective inhibitors of leukocyte infiltration and prevent tissue damage in several animal models of inflammation.[2] Inhibition of VLA-4 may reduce the migration and/or activation of cell types important to sustaining a prolonged inflammatory response. VLA-4 inhibitors may be considered for treatment of asthma,[3] multiple sclerosis,[4] and rheumatoid arthritis.[5]

We have previously reported the structure–activity relationship (SAR) of several series of sulfonylated dipeptide derivatives.[6–8] The pharmacokinetic (PK) profiles of **1** and **2** (Fig. 1) suggest that each has reasonably good oral bioavailability in several species (**1**: F%: 49% (rat), 27% (dog) 8% (rhesus monkey); **2**: F%: 43% (rat), 7% (rhesus monkey).[8] Based on these results and the potency enhancement found with substituted biphenylalanines, *N*-sulfonylated β-biphenylalanine-β-amino dipeptides were pursued. The hope was that the β-biaryl alanine scaffold would impart the same improved potency and pharmacokinetic properties seen in the β-phenyl-β-alanine series.[8]

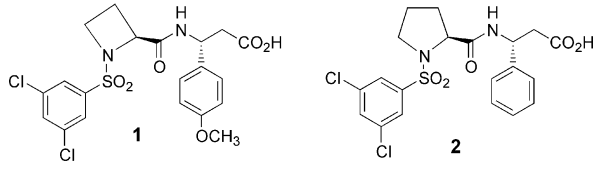

Figure 1. Inhibition of VLA-4 by β-phenyl-β-alanine derivatives.

Two routes were employed to synthesize the β-biaryl β-alanines. Boc-(*S*)-4-hydroxyphenyl glycine methyl ester was treated with triflic anhydride and homologated with an aryl boronic acid. (Scheme 1). The ester was then hydrolyzed, the free acid treated with disobutylchloroformate and reacted with diazomethane to

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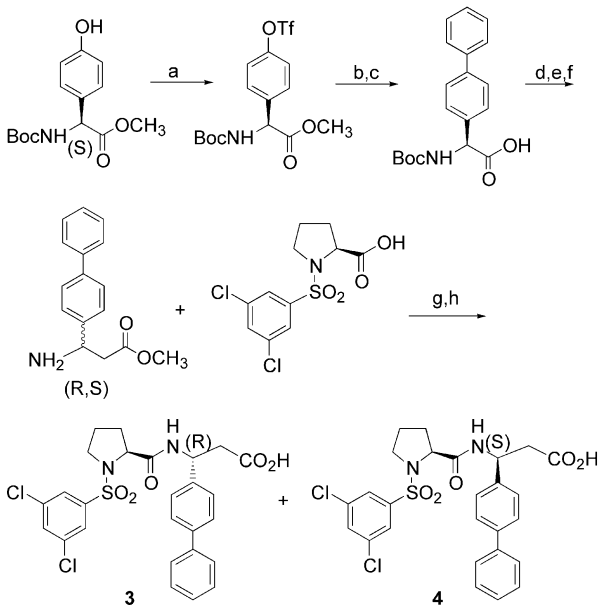
PII: S0960-894X(02)00460-2



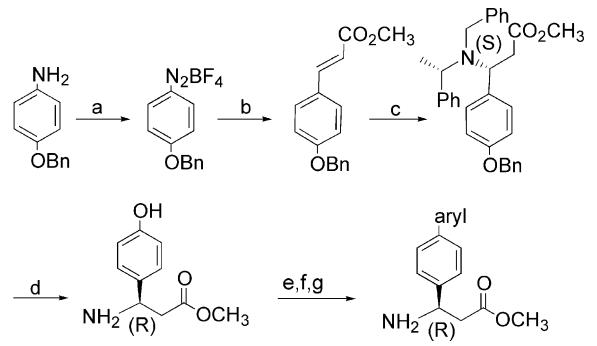
Scheme 1. (a) Triflic anhydride/Et_3N, CH_2Cl_2 0 °C, 2 h 85%; (b) phenylboronic acid, $Pd(PPh)_4$, K_2CO_3, (toluene/EtOH, 4/1) 60–90 °C, 2–3 h 75%; (c) LiOH, ($EtOH/H_2O$), H^+; (d) (i) isobutylchloroformate, Et_3N, (ii) diazomethane, Et_2O; (e) silver benzoate, Et_3N; (MeOH, dioxane, 0 °C, 2 h, 45% overall; (f) HCl (g), EtOAc, 0 °C 1 h, 95%; (g) PyBop, iPr_2NEt, CH_2Cl_2, 0 °C to rt; (h) (i) LiOH, $EtOH/H_2O$, rt, (ii) H^+, 60% overall.

form the diazoketone to effect the Arnt–Eistert reaction. The subsequent Wolff rearrangement with silver benzoate in dioxane/methanol afforded the Boc-3-(*R*)-amino-3-(4-biphenyl)propionic acid, methyl ester. Unfortunately, substantial quantity (~25%) of the undesired (*S*)-isomer was also obtained resulting from the unexpected isomerization of the stereogenic center. The unseparated isomeric mixture was carried through the reaction sequence by coupling the deprotected biaryl ester to (*N*)-(3,5-dichlorobenzenesulfonyl)-2-(*S*)-proline.

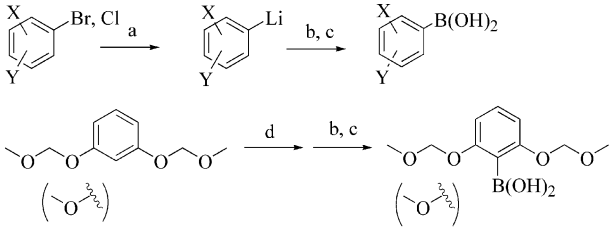
Diasteroisomers **3** and **4** were separated by flash column chromatography. This approach has two limitations. The first is the Suzuki coupling of our desired aryl boronic acid occurs prior to the Arnt–Eistert homologation sequence, limiting the synthetic flexibility for variations in that ring. The second is the partial isomerization of the β-carbon of the biaryl amino acid. A more flexible and efficient approach was developed.

A chiral synthetic strategy was employed by starting with (*R*)-β-4-hydroxyphenylglycine[9] (Scheme 2). This method gave high stereoselectivity for the Michael addition (>98% d.e.) and an excellent overall yield for the protected amino acid triflate. The triflate was subsequently used in the Suzuki coupling reaction with a wide variety of aryl boronates to form the desired substituted β-biaryl-β-amino acid intermediates. These β-aminoesters were used as before to obtain the test compounds **5**–**10**.

The aryl boronates were obtained from commercial sources or synthesized by metal halogen exchange of the aryl halide with *n*-butyl lithium and treatment with methyl borate. Some of the 2,6-dioxygenated arylboronic acids were synthesized by directed lithiation (*s*-BuLi or PhLi) of the parent arene (Scheme 3).



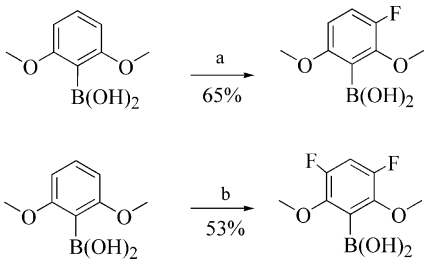
Scheme 2. (a) (i) 48% HBF_4, EtOH, (ii) isoamyl nitrite 5 °C; (b) methyl acrylate, 10% Pd/C, MeOH, reflux 3 h, 70%; (c) (i) 2 equiv (*S*)-(−)-*N*-benzyl α-methylbenzyl amine, 2 equiv *n*-BuLi, −78 °C, 30 min, (ii) NH_4Cl (aq), 66%; (d) 10% Pd/C, H_2, (50 psi), MeOH, 93%; (e) $(BOC)_2O$, DIEA; (f) triflic anhydride/Et_3N, CH_2Cl_2 0 °C, 2 h 85%; (g) phenylboronic acid, $Pd(PPh)_4$, K_2CO_3, (toluene/EtOH, 4/1) 60–90 °C, 2–3 h 75%.



Scheme 3. (a) *n*-BuLi, THF, −78 °C; (b) $B(OCH_3)_3$; (c) 2 N HCl; (d) PhLi, ether, 0 °C, 18 h.

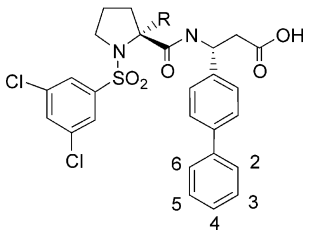
The directing ligand in the ring lithiation reaction is the methoxy group. Lithiation ortho to the directing group was generally obtained in good yield when it was effected at −78 °C. With examples **11** and **12**, we raised the temperature to 0 °C to accelerate the metallation process at the expense of a lower yield. The 2,6 bis-phenol **34** was protected as the bis-methoxymethyl ether since ortho metallation and acid deprotection of the MOM protecting group were easily achieved. The same sequence was used for the monophenol derivative **33**. We discovered that one could directly mono- and difluorinate the 2,6-dimethoxybenzeneboronic acid with the solid phase supported fluorinating reagents SelectFlor® (Scheme 4).[10]

The inhibition of binding of a soluble ^{125}I-VCAM-Ig fusion protein to VLA-4 on suspended Jurkat cells[6] is



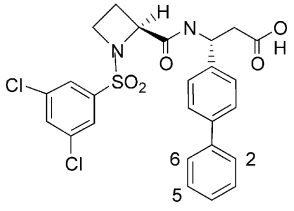
Scheme 4. (a) 1.1 equiv SelectFlor®, 22 °C, 24 h, CH_3CN; (b) 3.0 equiv SelectFlor®, 22 °C, 24 h, CH_3CN.

Table 1. Inhibition of VLA-4 by substituted *N*-sulfonyl-prolyl-β-amino acids (IC_{50}, nM)



No.	R	2	3	4	5	6	VLA-4
3	H (*R*)	—	—	—	—	—	0.8
4	H (*S*)	—	—	—	—	—	196
5	H (*R*)	OCH_3	—	—	—	—	0.6
6	H (*S*)	OCH_3	—	—	—	—	5.4
7	CH_3	OCH_3	—	—	—	—	10.0
8	H	CN	—	—	—	—	0.5
9	H	OCF_3	—	—	—	—	1.1
10	CH_3	OCF_3	—	—	—	—	7.1
11	H	OCH_3	—	—	OCH_3	—	0.2
12	CH_3	OCH_3	—	—	OCH_3	—	2.2
13	H	OCH_3	—	—	F	—	0.5
14	CH_3	OCH_3	—	—	F	—	17.5
15	H	OCH_3	—	—	—	F	0.5
16	CH_3	OCH_3	—	—	—	F	3.5
17	H	OCH_3	—	F	—	—	1.4
18	CH_3	OCH_3	—	F	—	—	38.7
19	H	OCF_3	—	F	—	—	2.7
20	CH_3	OCF_3	—	F	—	—	40.0
21	H	OCH_3	F	—	—	—	1.5
22	CH_3	OCH_3	F	—	—	—	31.4
23	H	F	OCH_3	—	—	—	1.7
24	CH_3	F	—	OCH_3	—	OCH_3	8.4
25	H	H	F	—	OCH_3	—	6.0
26	CH_3	H	F	—	OCH_3	—	88.8
27	H	OCH_3	—	—	—	OCH_3	0.13
28	CH_3	OCH_3	—	—	—	OCH_3	0.48
29	CH_3	OCF_3	—	—	—	OCH_3	1.15
30	H	OCH_3	—	—	F	OCH_3	0.2
31	CH_3	OCH_3	—	—	F	OCH_3	1.36
32	CH_3	OCH_3	F	—	F	OCH_3	1.18
33	CH_3	OH	—	—	—	OCH_3	0.40
34	CH_3	OH	—	—	—	OH	0.42
BIO-1211							0.13
TR-14035							0.11

Table 2. Inhibition of VLA-4 by substituted *N*-sulfonyl-azetidinyl-β-amino acids (IC_{50}, nM)



No.	2	5	6	VLA-4[a]
35	OCH_3	—	—	0.81
36	OCH_3	—	OCH_3	0.19
37	OCF_3	—	OCH_3	0.19
38	OCH_3	F	OCH_3	0.14
39	OH	—	OH	0.20
40	$(CH_2CH_2)CHO$	—	$(CH_2CH_2)CHO$	0.31

[a]^{125}I-VCAM-Ig.

summarized in Table 1. Several trends are noteworthy. First, the (*R*)-configuration of the substituted β-biaryl β-amino acid diastereomer is the more active derivative in the series (**3**,**4** and **5**,**6**). This corresponds to the same orientation as the substituted *N*-sulfonylated-prolyl-biphenylalanine derivatives in the α-amino acid series.[6] The 3, 4, and 5-monofluorinated pairs **21**–**22**, **17**–**18**, and **13**–**14**, respectively, were less potent than the parent compounds **6** and **7** while **15**–**16** were slightly more potent than the parent compound. The α-methyl-(L)-proline derivatives of each matched pair is substantially less potent than the des-methyl analogue. The difference between matched pairs is more pronounced in this β-series (7- to 40-fold) than in the α-amino acid series (2- to 5-fold).[6] The difference in potency between cognate pairs in this β-amino acid series becomes much smaller in the case of the 2,6-dioxygen substituted biaryl derivatives. The 2,6-dimethoxybiaryl pair **27** and **28** are among the most potent derivatives in the series as they are in the α-amino acid series. The monomethoxy-hydroxy and bis-hydroxy derivatives **33**, **34** are equipotent to the bis-methoxy congener **28**. The 2,6 dioxogen distribution is critical in achieving potency. Ring fluorinated derivatives **13**, **15**, **17**, and **21** as well as the trifluoromethoxy analogue **19** show only a small shift in binding potency from the corresponding hydrogen analogues. Binding data for two compounds described in the literature as a potent, selective VLA-4 antagonist (BIO-1211)[3] and a dual $\alpha_4\beta_7$/VLA-4 antagonist (TR-14035)[11] are included in Table 2 for comparative purposes. All of the derivatives in Table 1 show >500-fold specificity for $\alpha_4\beta_1$ over $\alpha_4\beta_7$ (data not shown).

Based on these results, a number of *N*-arylsulfonyl-L-azetidinyl derivatives were synthesized to compare binding potencies with selected prolyl derivatives. These results are shown in Table 2. The proline-azetidine pairs **5**,**35** and **27**,**36** were about equipotent in VLA-4 binding. All of the 2,6-dioxo species in Table 2 showed excellent binding to the receptor and at least >500-fold specificity for $\alpha_4\beta_1$ over $\alpha_4\beta_7$.

The pharmacokinetic properties of selected compounds were also determined (Table 3). Generally, the compounds had low to moderate oral bioavailability and moderate to fast plasma clearance rates. Several trends are apparent in the proline paired sets **13**–**28**. The des-methyl proline derivative are cleared from circulation much faster than the α-methyl analogues. Metabolism studies concluded that hydrolysis of the amide bond by circulating peptidases does not occur to any substantial degree. The bioavailability of the α-methyl proline analogues of each paired set was substantially greater than that of the des-methyl proline derivatives.

Most of the compounds were excreted into the bile as intact parent or as the conjugated acyl-glucuronide.[12] The low clearance, along with the very low volume of distribution, suggests that high plasma protein binding may be a significant contributing factor to the observed PK profile. Many of the compounds in Table 3 were found to be tightly bound to plasma proteins (>99.9%; data not shown). The same PK profile is seen with the

Table 3. Pharmacokinetic parameters[a] of selected compounds

No.	F[b] (%)	Cl_p (mL/kg/min)	*V*dss (L/kg)	$t_{1/2}$[c] (h)
1	43.2	22.1	1.5	1.1
4	8.9	14.6	0.61	1.8
5	21.6	1.1	0.08	1.3
13	13.9	13.1	0.58	3.0
14	19.4	2.3	0.08	1.2
15	12.2	16.3	0.19	0.7
16	42.0	2.3	0.13	1.3
17	8.2	14.6	ND[d]	ND
18	55.4	1.5	0.15	1.7
25	19.6	48.1	ND	ND
26	38.7	2.5	0.12	0.9
27	7.3	51.6	1.4	1.8
28	25.7	1.7	0.1	1.8
28[e]	1.0	10.4	0.26	ND
29	45.1	24.9	1.1	1.6
34	2.6	2.3	0.08	3.2
35	21.9	5.0	0.12	2.4
35[e]	1.0	22.1	0.25	ND
36	18.0	13.9	0.48	0.9
36[e]	1.0	21.7	0.37	0.7

[a]Sprague–Dawley rats.
[b]Dose: 1 mg/kg iv; 2 mg/kg po.
[c]$t_{1/2}$ = plasma half-life$_{(0-8\,h)}$.
[d]ND = Not determined.
[e]Rhesus monkey. Dose: 0.75 mg/kg iv; 1.5 mg/kg po.

azetidine derivatives in Table 2. For compounds with long plasma half-lives (**13**, **34**, and **35**), the plasma concentration in rats versus time curves (AUC) exhibit an initial rapid drop in concentration followed by low ($\sim$1 nM) but sustained circulating plasma drug levels in vivo.

In summary, *N*-sulfonylated dipeptide β-biaryl-β-amino acid derivatives are potent and specific antagonists of VLA-4 and compliment the corresponding substituted biphenylalaine analogues. The 2,6-dioxygenated biaryl substitution pattern is important for optimizing potency in both the azetidine and the proline series. The variable oral bioavailability, clearance and poor distribution may be directly attributed to the binding of these peptide-like compounds to circulating plasma proteins.

Acknowledgements

The authors are grateful to Zhen Wang and Junying Wang for formulation and mass spectral analysis and to Marcie Donnelly, Ken Vakerich and Chris Nunes for dosing of animals for pharmacokinetic evaluation.

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12. Results to be published in due course.